UNIWERSYTET MEDYCZNY IM. KAROLA MARCINKOWSKIEGO W POZNANIU

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Poznań, 15.01.2026

Department/Institution: Department of Oncology
Reviewer: Dariusz Iżycki
Academic Title: Professor of Immunology and Oncology UMP

REVIEW OF DOCTORAL THESIS

Candidate
Mariam Ibrahim

Title of the dissertation: *Intergenerational and transgenerational effects of epigenetic factors applied in early developmental stages – insights from in ovo model*

Field of study: health sciences

The doctoral dissertation submitted by Ms. Mariam Ibrahim addresses a current, scientifically demanding research problem, situated at the intersection of nutritional programming and epigenetics, developmental biology, and reproductive biology. The work focuses on the inter- and transgenerational consequences of early-life exposure to bioactive compounds, namely a synbiotic preparation and choline, using a controlled experimental condition in a well-established, multigenerational *in ovo* model.

The relevance of this topic is justified by the growing recognition that environmental and nutritional factors acting during critical developmental windows may shape long-term health outcomes, not only in directly exposed individuals but also across subsequent generations. The field of epigenetic studies remains scientifically challenging and is still conceptually evolving. Despite increasing interest in this field, experimental studies integrating multigenerational design with high-throughput genomic analysis remain scarce,

particularly those integrating transcriptomic and epigenetic approaches. In this context, the thesis fills an important knowledge gap and contributes to the field development.

The dissertation is clearly structured as a monothematic cycle of 4 publications (Papers I–IV) and one complete manuscript draft (Paper V), followed by a synthetic discussion and conclusions. The thesis has logically organized introduction, with a clear summary of methods and major results.

In chapter 3, the candidate convincingly demonstrates that the individual papers are not independent outputs but form together an interconnected components of a wider, coherent research project, guided by clearly defined hypotheses and objectives. High level of conceptual and thematic coherence is a key strength of the presented dissertation. All included studies are explicitly linked to the central research aim: to investigate whether prenatal exposure of a developing organism and germline tissue to factors considered epigenetic, induces changes in somatic and germline tissues. Paper I establishes the theoretical framework for the research conducted, Paper II explores the germline model, and Papers from III–to V systematically address somatic and germline transmission of the effects of prenatally applied epigenetic factor, across generations. Altogether, the published papers (II- to IV) and the drafted manuscript (V) provide a relevant and complex research outcome that allowed verification of four major hypotheses stated in the dissertation. The rationale for tissue selection (cecal tonsils, cecal mucosa, embryonic blood and gonads) is explained, and the use of primordial germ cells (PGCs) as a model system is justified.

The candidate performed an advanced, bioinformatic exploration of multiple tissues in multiple generations (F1–F4), comparing single versus repeated exposures to prenatal epigenetic stimulus.

Such complexity of a study design is rarely achieved in doctoral research and reflects high methodological competence and reflects the scientific ambition of the candidate.

The thesis, typically in this type of dissertation, incorporates the published, peer-reviewed versions of the monothematic cycle of the articles, reflecting the scientific maturation process of the candidate. The candidate is a leading author of all the papers submitted as a part of her thesis, which demonstrates her ability to engage in high-level scientific discourse, and respond to criticism being part of peer- revision.

The included bibliography is extensive and well curated, comprising several hundred references. The majority of cited works originate from high-quality international journals published within the last 5–10 years, particularly in the fields of epigenetics, developmental biology, nutrition, and animal models of health research.

Stating conclusions, the candidate avoids unjustified generalizations and correctly frames her work as an investigation of **transcriptomic effects across generations,** rather than a definitive proof of molecular inheritance mechanisms.

Regarding the language and editorial quality, the dissertation is written in very good scientific English, with precise terminology and consistent style.

The literature review is comprehensive, critical, and well-structured. The candidate does not merely summarize previous findings but identifies unresolved questions, particularly regarding the persistence, tissue specificity, and non-linear nature of transgenerational effects.

Research methodology involved typical, appropriate molecular tools and *state-of-the-art* bioinformatic analysis for genomic studies. The quality of genetic material used and analytical pipelines were validated and controlled prior to use in analyses, which is shown in an extensive set of supplementary material in candidate's publications, clearly explaining the methodological approach and confirming her data quality. The combination of RNA-seq and targeted RT-qPCR validation strengthens the reliability of her findings.

Statistical approaches were correctly selected and properly applied. The candidate demonstrates awareness of the limitations imposed by sample size and biological variability, and her interpretations remain cautious and evidence-based.

The results are presented clearly through well-designed figures and tables.

The most significant findings in the thesis demonstrated that:

-prenatal stimulation of embryo with epigenetic factor using the *in ovo* model induced intergenerational (F2) and transgenerational (F3–F4) transcriptomic effects, which were tissue-specific and generation-dependent

-repeated exposure does not result in simple cumulative amplification, indicating non-linear inheritance patterns of the selected effects

These observations substantially advance current understanding of epigenetic programming. The discussion chapter is mature, integrative, and critical, finely organized in 7 subchapters including discussion of major observations selected by the candidate. The candidate openly discusses limitations of her study (e.g., incomplete methylome coverage), and avoids overinterpretation. The thesis identifies future research directions, including deeper epigenomic analyses and functional validation.

Overall, the doctoral dissertation submitted by Ms. Mariam Ibrahim represents an original, ambitious, and methodologically relevant contribution to health sciences using an accessible, multigenerational study model. The candidate demonstrates advanced theoretical knowledge and ability to conduct multigenerational analyses and complex interpretation.

Therefore, in my opinion the dissertation fully meets the conditions specified in Article 187, Sections 1–4 of the Act of July 20, 2018, on Higher Education and Science.

Recommendation

Hereby, I submit to the Disciplinary Council of Health Sciences at Nicolaus Copernicus University a motion to admit Ms. Mariam Ibrahim to the further stages of doctoral proceedings.

Poznań 15.01.2026

Dominik Lyoka

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